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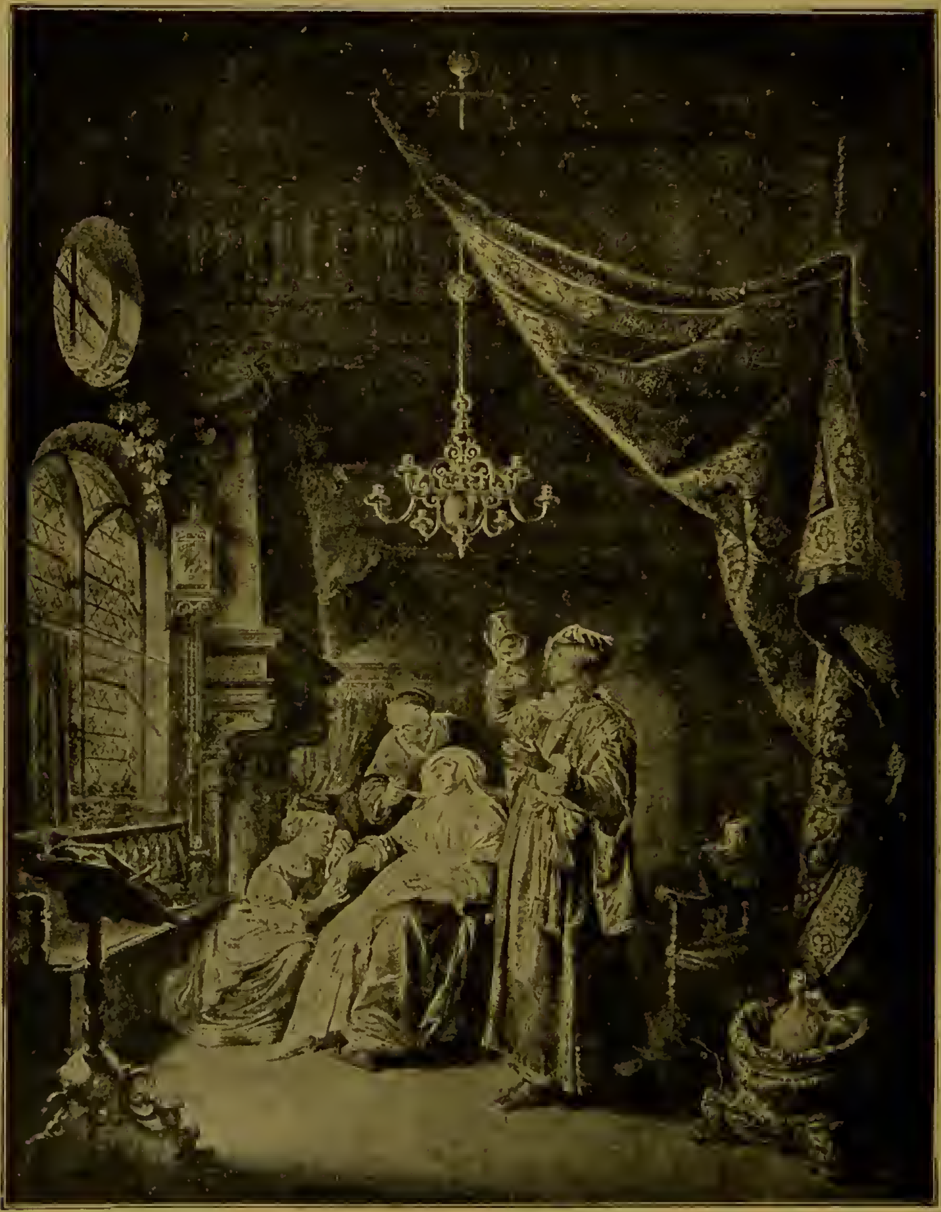

St. Bartholomew's Hospital.

NOTES

ON THE

EXAMINATION OF URINE

FOR HIS CLINICAL CLERKS.

By SIR LAUDER BRUNTON, M.D.

PART I.

NOTES ON THE EXAMINATION OF URINE.

By SIR LAUDER BRUNTON, M.D.

MULTIPLICATION of means of research is sometimes attended with the disadvantage that it leads to neglect of obvious conditions, and chemical analysis is sometimes resorted to by the student to the exclusion of observations on the colour, appearance, and sediments of the urine. The beautiful picture, by Gerard Dow, which hangs in the Louvre, shows a physician examining the urine of a dropsical woman. By holding it up to the light he could determine its colour and see whether it was clear or contained a sediment; by shaking it he would see whether any permanent froth was formed. The formation of froth was noticed by Hippocrates, who said that when bubbles settled on the surface of the urine they indicated disease of the kidneys, "and the complaint will be protracted." Although the physician painted by Gerard Dow had not the same knowledge of the pathology of dropsy that we have, yet by imitating his practice we may learn a good deal about the probable cause of the patient's illness without resorting to any chemical methods of analysis, although these ought afterwards to be applied. Thus if we shake the urine and find that permanent froth settles we know that it contains albumen or bile. If the froth is yellow we decide in favour of bile, and if the froth is white, albumen is present. If the urine is pale the albumen is probably due to renal disease, whereas if the urine is dark the albumen is probably due to cardiac disease. If a thick reddish precipitate be present in addition to the albumen it generally means that the liver is more or less involved, either secondarily to cardiac disease or primarily from cirrhosis. The distribution of the dropsy here tends to guide us, for if the abdomen be distended either without swelling of the legs, or disproportionately to swelling of the legs, then cirrhosis is probably present; whilst if the swelling be more marked in the legs than in the abdomen, it is probably due to cardiac disease. It is well to cultivate the habit of looking at such signs as these as well as to learn the more accurate methods of chemical and histological examination which are required in order to make an accurate diagnosis.

NORMAL URINE.

(1.) *Constituents.*—Normal urine contains the nitrogenous waste of the body arising from the metabolism of proteids which either form part of the tissues or are absorbed from the intestine and, after conversion into urea in the liver or elsewhere, are quickly excreted.[1]

This nitrogenous waste occurs in the urine chiefly in the form of urea, although a small proportion occurs as uric acid, and a still smaller quantity in the form of kreatinin, &c.

(2.) In addition to urea, &c., the urine contains inorganic salts, phosphates, chlorides, and sulphates of sodium, and to a small extent of ammonium and potassium, of calcium and magnesium.

(3.) The urine contains water, which dissolves the substances already mentioned, and which thus enables them to be excreted from the body.

The urine also serves to remove excess of water from the body when much water has been drunk.

(4.) It also contains pigments on which its colour depends.

Measurement of the Quantity of Urine.—The whole of the urine passed in 24 hours is usually collected in hospitals in a large graduated glass. When no such glass is available the urine passed may be poured each time into a Winchester quart or other vessel, and afterwards measured out by means of any measure available. Should this measure be small, it will save trouble to paste a slip of white paper vertically on the outside of the Winchester quart, and then pour into it measured quantities of water, say an ounce at a time, and mark off these quantities on the paper. The Winchester quart then serves to measure the urine as well as to contain it.

If the collection be made, let us say from 8 A.M. one day to 8 A.M. the next day, the bladder should be emptied at 8 and the urine thrown away, as it has been excreted before the time of observation begins.

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[1] After a meal containing much proteid the whole of the part absorbed is excreted in the form of urea within a few hours.—Schäfer, *Text-Book of Physiology*, vol. i, p. 904.

Next day the bladder should be emptied at 8 A.M., and this urine completes the quantity secreted in 24 hours.

In order to avoid the loss which is apt to occur when the bowels are opened, the patient should use a urinal at the same time as the closet or bed pan.

Concentration of Urine.—The quantity of solids daily passed in the urine varies according (1) to the amount of tissue metabolism and (2) the quantity of soluble salts and nitrogenous waste due to absorption of salts and proteids from the intestine.

But the quantity of solids is much more constant than the quantity of water, which varies in wide limits according to the amount of liquid drunk and to the loss of water from the skin or bowels by sweating, purgation, or diarrhœa.

The concentration of the urine therefore depends chiefly on the greater or less amount of water it contains. When abundant it will be less concentrated, and when scanty it will be more concentrated in health.

Specific Gravity of Urine.—The concentration of urine is ascertained by taking its specific gravity.

This is done by means of the urinometer. This instrument is usually kept in hospitals in a glass of clean water. In using it the urine should be put into a glass (best cylindrical), and the urinometer put gently into it, taking care that it does not stick to the side of the vessel nor rest upon the bottom.

If it sticks to the side it must be pushed into the middle of the glass. If it rests on the bottom more urine must be added. If there be not enough urine, sometimes one may get the urinometer to float by putting the end of a test-tube into the urine beside the urinometer. If this is insufficient the urine must be measured in a small glass, and then once, twice, thrice, or four times its bulk of water added to it.

The specific gravity of the mixture is then taken, and if its own bulk of water has been added to the urine so that its volume has been doubled, the specific gravity found must be doubled to get that of the original urine. If twice its bulk has been added so that the volume of the mixture is treble that of the original urine, the specific gravity observed must be multiplied by three, and so on.

The urinometer is graduated for urine at the ordinary temperature of the air, 60° F. (*i.e.*, about 15° C.). When the urine is warm its specific gravity is lower than when cold, so that if one clerk takes the specific gravity of a certain urine directly it is passed, and another takes it after the urine is cold, they will disagree about the result.

The ordinary specific gravity of urine is about 1020 or 1022, but it may vary in health from 1015 to 1035. Whenever it approaches these extremes, however, suspicion should be aroused, and careful examination of the urine made and careful inquiries made in order to be certain that no disease is present.

Reading of the Urinometer.—In order to read the urinometer accurately any air bubbles around its stem should be removed by a piece of filter-paper. It should then be held level with the eye and the lower edge of the liquid read off.

When the specific gravity of the urine is nearly normal an exact reading is less important, for practically it makes no difference whether the specific gravity be 1021, 1022, or 1023. But it makes a considerable difference if the specific gravity is low, or if we wish to use the specific gravity as a means of estimating solids in the urine.

Rough estimation of Solids from the Specific Gravity of Urine.—At best this can only be approximate, for urea and salts have different powers of raising the specific gravity. Thus a 10 per cent. solution of sodium chloride has a specific gravity of 1073, whilst a 10 per cent. solution of urea has only a specific gravity of 1028.[1]

The amount of solids is estimated by multiplying the last two figures of the specific gravity by 2·33. This gives the amount per 1000, or, in other words, the number of grammes of solids in a litre of urine. In order to get the percentage this must be divided by 10.

Thus, if the specific gravity be 1022, the amount of grammes per litre will be 22 × 2·33 = 51·26. The percentage will be one-tenth of this, viz., 5·126. To convert the percentage into grains per oz., multiply it by 4·375. Thus 5·126 × 4·375 = 224 grains per oz. The smaller fractions may be neglected in such a rough method, for the data are so uncertain that to carry out the product to a number of decimal places gives an appearance of exactitude which is simply delusive. This is all the more evident when one remembers that others have recommended the two last figures of the specific gravity to be multiplied by 2·2[1] instead of 2·33.

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[1] Schäfer's *Text-Book of Physiology*, vol. i, p. 574.

As an exercise in this it may be well to try how far concordant results are obtained by multiplying the last two figures of the specific gravity actually obtained after diluting a specimen of urine once, twice, and thrice by 2·33, and then by the number of times the urine has been diluted. As the total solids are unchanged the results ought to be absolutely identical, and any variations are due entirely to experimental error.

Colour of the Urine.—In healthy urine this is chiefly due to a substance named urochrome. When the concentration of the urine is about normal, as indicated by a specific gravity about 1022, it usually is of a straw colour. When more dilute it is paler, and when more concentrated it is darker. In abnormal conditions other pigments may appear, or the normal pigment may be diminished so that the colour no longer corresponds to the specific gravity.

Reaction of the Urine.—It is generally more or less acid. The acidity is generally tested by dipping a piece of blue litmus paper into the urine and noticing the reddening, which indicates acidity. The amount of reddening is roughly taken as an indication of the amount of acidity. Should no reddening occur then put in a piece of red litmus paper and see whether it becomes blue or not. If it does this indicates that the reaction is alkaline. Sometimes the urine presents an amphoteric reaction, *i.e.*, it turns blue litmus red and red litmus blue. The reason of this is that the urine does not contain free acid, but owes its acidity to dihydrogen or acid sodium phosphate (NaH_2PO_4). Both this salt and monohydrogen phosphate (Na_2HPO_4), which has an alkaline reaction, exist in the urine. When the former predominates the urine has an acid reaction, and when the latter predominates the reaction is alkaline. When they are nearly evenly balanced the reaction is amphoteric.

If the urine be strongly acid the addition of a few drops of liquor potassæ or liquor sodæ will increase the proportion of monohydrogen phosphate and render the reaction amphoteric or alkaline according to the amount added ($NaH_2PO_4 + NaHO = Na_2HPO_4 + H_2O$). The addition of acid to a neutral or alkaline urine on the contrary will render it acid. $Na_2HPO_4 + HCl = NaH_2PO_4 + NaCl$.

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[1] Gowland Hopkins, Schäfer's *Text-Book of Physiology*, vol. i, p. 574.

Deposits in Urine.—The two chief deposits in the urine of healthy persons are urates and phosphates.

The disposition of urates occurs in urine which is *concentrated* and rather *strongly acid.*

Phosphates are deposited when the urine is only faintly acid, neutral or alkaline.

Urates.—Urates are readily soluble at the temperature of the body, and, therefore, the urine which deposits them is clear when passed, but becomes muddy and throws down a deposit when it becomes cold.

To show the effect of (1) warmth, (2) dilution, and (3) reaction, take some urine containing urates and divide it into three parts.

(1.) Warm the first part and it will become clear. Cool it under the tap and as it cools it will again become turbid.

(2.) Dilute the second with its own bulk of water. It will probably not become clear, but if it is warmed like the first it will probably remain clear on cooling, the added quantity of water being sufficient to keep the urates in solution.

(3.) To the third add a few drops of liquor potassæ or liquor sodæ. It will probably not clear up at once, but will remain clear after warming like the second.

Phosphates.—About 75 per cent. of the phosphoric acid in urine is combined with sodium ammonium and potassium. The *alkaline* phosphates, as these salts are termed, are very soluble in water and never form deposits. The remaining 25 per cent. of phosphoric acid is combined with calcium and magnesium to form the so-called earthy phosphates. The *earthy* phosphates are soluble in acid urine, but when the reaction becomes alkaline they become insoluble and fall as a deposit.

Add to some urine in a test-tube enough liquor potassæ to make it alkaline and let it stand awhile. It will become muddy and a deposit of phosphates will fall.

To another portion add only enough liquor potassæ to render the urine neutral. Boil the urine and phosphates will be deposited. The reason usually given for this is that, by boiling, some CO_2 is drawn off, and the liquid becoming more alkaline the phosphates are deposited.

Add a few drops of acetic acid to the turbid urine and the deposit of phosphates will redissolve.

See solubility of acalc. phospt. in hot water.

CAUSES OF VARIATION IN THE URINE IN HEALTH.

Quantity.—The quantity depends (1) on the composition of the blood, and (2) on the pressure of blood in the renal glomeruli.

The blood becomes more watery, and the urine increases in quantity when much liquid—such as water, soda-water, beer, tea, coffee, or soup—has been drunk. It becomes less watery and the urine diminishes when little fluid is taken or when much water is lost from the skin by profuse sweating or from the bowels by purgation or diarrhœa.

The tension is increased in the renal glomeruli when the capillaries of the skin become contracted by exposure to cold. Such exposure has a double action, for it not only increases the arterial tension in the glomeruli, and this increases the urine, but it lessens by loss of water from the skin, and tends in this way also to increase the urine.

In some experiments which I made on myself in 1865, but which I did not publish, I found that on a cold day following several hot ones, the quantity of urine became doubled.

Emotion has a great effect in increasing the amount of urine, and the excitement of an examination is well known to stimulate the kidneys of the man undergoing it. It also stimulates the circulation, and it is interesting to notice that during examination, a student's ears have a deep red flush from dilatation of the capillaries, while the temporal arteries are dilated and prominent and throb so violently that the movement may be seen from a considerable distance.

Specific Gravity and Colour.—These are usually inversely as the quantity.

Reaction.—Much meat in the dietary, much muscular exercise and fasting increase the acidity. Vegetable diet, sedentary occupation, and digestion lessen acidity.

When much acid has been secreted into the stomach as gastric juice the blood and urine tend to become more alkaline.

After the acid has been reabsorbed the urine recovers its normal acidity. This tendency to alkalinity in the urine during gastric digestion is sometimes called the "alkaline tide." This is most marked about 11 o'clock in the forenoon during the digestion of breakfast, and not unfrequently it is so decided that the urine is quite milky when passed. This sometimes gives rise to great anxiety on the part of the patient, and even doctors sometimes think it betokens great loss of phosphates from the body. As a matter of fact such urine may contain considerably less phosphoric acid than clear urine, and the appearance of a deposit is merely a question of acidity. On the addition of a few drops of acetic acid the turbidity will clear up.

ABNORMAL CONDITIONS OF URINE.

As I have already mentioned, the quantity of urine may be greatly increased by drinking fluids, exposure to cold, &c., but such an increase is usually only temporary, and is often succeeded by a temporary diminution, so that the average quantity taken over a few days is nearly normal. When the quantity is more or less permanently increased or diminished the condition becomes abnormal, and its cause must be investigated.

ABNORMAL QUANTITY.

A permanent increase in the quantity of urine suggests a permanent change either (1) in the composition of the blood, or (2) in the pressure in the glomeruli, or (3) in both.

(1.) An alteration of the blood occurs in diabetes mellitus where glucose is present in it in abnormal quantity and passes from it into the urine.

(2.) An increase in the glomerular pressure occurs in (*a*) gouty kidney where the urine is pale, of low specific gravity, and may contain a trace of albumen. In this condition the amount passed during the night undergoes a notable increase so that the patient has often to rise several times to make water. The cause of this is not known, but I think it may be due to relaxation of vasomotor impulses from the central nervous system to the renal vessels in a similar way as vessels elsewhere, in skin, &c., relax during sleep.

(*b*.) Alteration in the glomerular pressure is probably also the cause of the profuse flow of watery urine in diabetes insipidus.

(3.) In amyloid disease of the kidney the urine is abundant, pale, and contains much albumen. It is at present impossible to say how far the abundant secretion is due to change in the blood, in the circulation, or possibly in the kidney substance, or all of them together.

Abnormally scanty urine occurs (*a*) in fever, (*b*) in conditions of low-blood tension, such as fatty or dilated heart, or disease of the mitral valves.

Specific Gravity.—This is generally inversely to the quantity, and when this is not the case, *i.e.*, when the quantity is great and yet the specific gravity is high, glucose is usually present.

Colour.—This, like the specific gravity, is usually inversely as the quantity. When the specific gravity is low, and yet the colour is deep, some abnormal colouring matter—such as bile, blood, &c.—is likely to be present.

When the specific gravity is normal, but the colour is abnormally pale and no sugar is present, as in chlorosis, test for chromogens by filling a test-tube about one-third full of urine and letting a few drops of strong nitric acid run gently down the side of the tube to the bottom. A rose purple colour will usually appear.

Abnormal Smell of Urine.—When vesical catarrh is present the urine may decompose before it is passed, and it will then have an ammoniacal smell. Under some conditions not yet ascertained the urine may have a smell of herring brine (trimethylamine). In diabetes the urine has a sweetish smell, which is said to resemble new-mown hay, and when the disease is advanced and acetone is present, the odour may be fruity. Turpentine gives a smell like that of violets, and after asparagus has been eaten a very peculiar smell is observed in the urine. It is due to

ABNORMAL CONSTITUENTS OF THE URINE.

The most important are albumen, blood, pus, sugar, and bile. Both blood and pus contain a certain amount of albumen, and therefore if either of them be present in the urine there must be at least a trace of albumen present, but albumen may be present without either blood or pus.

Tests for Albumen.—The three tests most commonly employed to detect the presence of albumen are its precipitation—

(1.) By boiling.
(2.) By cold nitric acid.
(3.) By a saturated solution of picric acid, along with some citric or acetic acid.

When the result of any of these is uncertain, one or both of the others should also be applied.

(1.) *Test by Boiling.*—When a urine containing albumen is boiled, the albumen coagulates and forms either a haze or a more or less dense precipitate, according to the amount of albumen present. If the urine is turbid, a haze may not be perceptible, and so the presence of albumen might be overlooked. If the turbidity is due to the bacteria of commencing fermentation the urine must be filtered.

Usually, however, turbidity is due to urates or phosphates. If the turbidity is due to urates, fill the test-tube about half full of urine and warm the whole of it until the urates dissolve. Then lay hold of the lower end of the tube and heat the upper half of the urine in the flame until it boils. The advantage of heating only the upper half of the urine is that the lower serves as a standard of comparison, and thus slight changes may be detected which might otherwise pass unnoticed. If no cloud appears the urine is free from albumen, but before coming to this conclusion the test-tube should be held against a dark background whilst the light strikes upon it. If a cloud appears add a drop or two of acetic acid. If the cloud clears up completely it is not albuminous but phosphatic. Phosphates are very rarely precipitated by boiling in urine which deposits urates, for urates are deposited in acid urine and phosphates are soluble in such urine and are usually precipitated by heat only from neutral or alkaline urine. I have however, sometimes seen phosphates precipitated on heating urine which had been turbid with urates.

If the urine is turbid from phosphates add a few drops of acetic acid and then heat as before.

There is some, but not much danger of producing acid albumen by acetic acid, but if nitric acid be used acid albumen may easily be formed and no precipitate produced on boiling, although albumen be present. To show this add a few drops of nitric acid to about half a test-tube full of urine. Throw it out and without washing the tube half fill it with albuminous urine. On then boiling it, very probably no precipitate will occur.

(2.) *Cold Nitric Acid.*—In applying this test nitric acid and urine are brought into contact with each other as far as possible without mixing. If albumen be present a white ring of coagulated albumen occurs at the place of junction. The test may be applied in a conical test-glass, in a glass cylinder such as is usually employed for the use of the urinometer, or in a test-tube. The acid may be poured in first and the urine gently poured on its surface, or the urine may be poured in first and the acid gently poured in after, so as to run down the side of the vessel and form a stratum below the urine. When the acid is poured in first the urine may be gently placed on the top of it by a pipette, which is less liable to disturb the acid and cause admixture than pouring the urine in. But the use of the pipette takes more time, and with care it is generally easy to pour the urine in without disturbing the acid.

Fallacies of the Nitric-Acid Test.—The white ring due to albumen appears *close* to the junction of the urine and acid. A cloud may appear higher up from the precipitation of uric acid.

In the urine of persons who are taking copaiba or other resins, a white cloud resembling that of albumen may appear, but it disappears on adding half its bulk of rectified spirit to the urine.

(3.) *Picric-Acid Test.*—A saturated solution of picric acid poured into urine usually causes a pale yellow precipitate, but it does not always do so unless it be used in very large quantity. If mixed with citric or acetic acid it precipitates albumen more readily. Instead of pouring the reagent into the urine, the process may be reversed and the urine poured drop by drop into the reagent. This plan has the advantage that the reagent is always in excess, and the failure above mentioned will not occur.

Fallacy of this Test.—If the patient is taking quinine a precipitate almost exactly like albumen will occur, but it disappears on heating.

Quantitative estimation of Albumen.—An old plan was to boil some urine after acidulating with acetic acid, and to set it aside for some hours.

According to the proportion of the height of the precipitate to the total column of urine, the quantity of albumen was spoken of as $\frac{1}{8}$, $\frac{1}{6}$, $\frac{1}{4}$, &c. This plan is now almost wholly replaced by the use of Esbach's albuminometer.

This consists of a graduated tube. Urine is poured into this up to a mark U, and reagent up to a mark R. The two are then shaken together and allowed to stand for 24 hours. The height of the precipitate is then read off on a graduated scale which gives the amount of dry albumen per 1000 parts of urine. To get the percentage amount divide this by 10. To get the number of grains per oz. multiply the percentage by 4·375, or the original number by ·4375. (There are 437·5 grains in an ounce.)

Precaution in using this Test.—If the urine is concentrated it is best to dilute it to a specific gravity below 1012, and when this is done the amount of albumen obtained must be multiplied by the number of times it has been diluted.

Tests for Blood.—The presence of blood in small quantity may be suspected if the urine has a smoky colour. In large quantities blood gives it a distinct red colour.

The urine containing blood will give the reactions for albumen, and microscopic examination of the sediment will show red corpuscles.

The test most commonly used for blood is the blue colour which it gives with tincture of guaiac and ozonic ether. In using this test put about an inch of urine into a test-tube; put in 3 or 4 drops of guaiac tincture, shake, then pour over it about $\frac{1}{4}$ to $\frac{1}{2}$ an inch of ozonic ether without shaking. A blue colour appears at this junction if blood be present.

Fallacies.—Saliva also gives this reaction; and if the patient has spit much into his urine a positive result may be obtained, although in reality the urine may be free from albumen.

Pus also gives a greenish blue colour.

If the patient is taking iodide of potassium a blue colour may appear, but it occurs more slowly, is of a somewhat greenish tint, and occurs throughout the urine and not at the junction only.

Test for Iodide in the Urine.—If the guaiac test gives an indication of iodides in the urine, it is advisable to make certain by applying another test.

(1.) Take about 3 inches of urine in a test tube, add to it 4 or 5 drops of fuming nitric acid and about ½ an inch of chloroform. Shake and allow the chloroform to settle. If iodides be present the nitric acid will set free iodine, and this will dissolve in the chloroform and form a *purple* solution at the bottom of the test-tube.

(2.) A little starch paste added to the urine along with a drop or two of nitric acid will give a blue colour. This is quite as good as the first test, but starch paste does not keep well, and it may not be at hand, while nitric acid and chloroform usually are.

Tests for Pus.—Urine containing pus gives indications of albumen and a greenish colour with tincture of guaiac. On boiling, this colour disappears.

The usual test is to pour off the urine from the deposit of pus or transfer some of the deposit by a pipette to a test-tube. Then add some liquor potassæ and the mixture becomes gelatinous and ropy, so that when poured from one test-tube to another it slips out of one and into the other like white of egg.

On microscopical examination the sediment is seen to consist of agglomerated leucocytes.

Tests for Bile.—One of the readiest ways of recognising bile in the urine is to notice the yellow stain it causes on the patient's shirt. By dipping a piece of filter-paper in it a similar stain may be noticed, and this is still more observable if a quantity of urine is allowed to pass through a filter. Shake the glass and notice that the froth is also yellow.

There are two tests commonly used for bile pigments: (1) iodine, and (2) nitric acid. Two are used for bile acids: (1) sulphur, and (2) sulphuric acid and sugar.

Bile Pigments.—On adding a few drops of tincture of iodine to urine containing bile a green colour is produced.

(2) *Nitric-Acid Test.*—This is often known as Gmelins' test. It depends on the fact that the products of oxidation of bilirubin have colours in the same order as the rainbow, so that we pass from yellow (bilirubin) to green (biliverdin), then to blue, next to purple, and last to orange. The latter products of oxidation of bilirubin are very unstable, and have

not been isolated. The green colour (biliverdin) is the characteristic one, and unless t is seen the other colours are not conclusive of the presence of bile.

This test may be applied by simply putting about 3 inches of urine into a test-tube, and allowing some fuming nitric acid to flow down the side. At the place of contact the green colour first appears, and then as oxidation advances the green travels higher up the tube, and below it appear successively blue, purple, and orange. If there is too much bile in the urine the colours are too dark to be seen well, and then the urine should be diluted before adding the nitric acid.

Another plan, which many prefer, is to pour a little of the urine on to a white plate and place a few drops of nitric acid near it. By gently inclining the plate the two liquids are brought together, and when they come in contact, if bile is present, the same rainbow-like play of colours occurs which has just been described. The nitric acid may be dropped from a pipette, but it is simpler to drop it from a bottle. Instead of putting the urine on a plate it may be made to wet a piece of filter-paper, and the nitric acid dropped on. If there is only a little bile in the urine a better result may be obtained by filtering a considerable amount of urine through it before applying the test.

Tests for Sugar.—The two chief tests for sugar depend on (1) its decomposition by fermentation with yeast and production of alcohol and CO_2; (2) its power of reducing cupric to cuprous hydrate in alkaline solution.

(1.) The yeast test may be applied by taking a piece of German yeast, about the size of the kernel of a hazel-nut, and mixing it well with sufficient of the urine which is to be tested to fill a test-tube. Partly fill a conical test-glass with the urine, and then, putting the thumb on the mouth of the test-tube containing the urine and yeast, invert it into the glass, only withdrawing the thumb when the mouth of the tube is under the urine. Support the tube so that it does not fall, and let it stand in a warm place for 24 hours. If sugar be present in the urine it will undergo fermentation, and more or less CO_2 will accumulate at the top of the tube.

Fallacies of this Test.—(1.) The yeast may be inactive. (2.) It may itself contain sugar.

(1.) In order to be sure that the yeast is active, another tube should be filled as above with yeast, and a solution of glucose instead of urine, and treated as before. If no CO_2 is found, the yeast is inactive.

(2.) A third tube should be filled with yeast and water or urine known to be free from sugar, and treated as above. If CO_2 is formed, then the yeast itself contains sugar.

If the yeast is defective in either of these ways it must be thrown away and some fresh yeast got. If it has already been tested and found good, the control tests just mentioned need not be applied.

Instead of the plan just described, the simple apparatus described by Claude Bernard may be used.

The advantage of the simple methods already described is that the apparatus is likely to be at hand, and can be easily made if more than one should be wanted for control experiments, and it is easy to replace if broken. Instead of a test-tube and glass, a bent and bulbous tube may be used, the long limb of which is filled with urine and yeast, and the CO_2 accumulates at the top.

Copper Test.—(2.) Take about 2 inches of water in a test-tube. Add a quarter to half an inch of liquor potassæ or liquor sodæ, then pour in two or three drops of a solution of cupric sulphate. A pale blue precipitate of cupric hydrate will fall, and will not redissolve on shaking the tube. Repeat this with urine free from sugar, and the result will be the same. Boil the mixtures containing water and urine, a black precipitate of cupric oxide will be formed in both.

Repeat the experiment with water, to which a little glucose has been added, and instead of the cupric hydrate falling as a precipitate, it will remain in solution, glucose having the power to keep it in solution.

Other substances besides glucose have this power. Repeat the experiment, using some Rochelle salts (tartrated soda), and the result will be the same.

Now boil the solution containing cupric hydrate with glucose in the one tube and with Rochelle salts in the other, and a very marked difference will be observed. The solution in Rochelle salts will remain unchanged, but that containing glucose will lose its blue colour, and a precipitate of yellow cuprous hydrate or red cuprous oxide will be thrown down in consequence of the glucose having reduced the cupric hydrate.

There are several modifications of this test which are known by the names of their authors.

(*a.*) Trommer's Test consists in adding liquor potassæ or sodæ and some solution of copper sulphate to urine, and boiling. If sugar is present the cupric hydrate is dissolved at first and is reduced on boiling. The test is therefore convenient enough if sugar is present in the urine, but if it is not, then the precipitate remains undissolved and is unpleasant to work with.

(*b.*) Fehling's Test avoids this discomfort by adding to the alkaline solution some Rochelle salts, so that whether sugar be present in the urine or not one does not get any precipitate in the cold. Fehling's solution consists of caustic soda, Rochelle salts, and cupric sulphate dissolved in water. If kept for a long time the solution is apt to undergo spontaneous change, with the result that when boiled it may undergo reduction, although no sugar be present. In order to avoid this risk it is advisable to boil such a solution for a few minutes before adding the urine. Another method of preventing change in the solution is to keep the solution of cupric sulphate in one bottle and that of the caustic soda and Rochelle salts in another, and only mix them at the time of using.

(*c.*) *Pavy's Test.*—About 40 years ago liquor sodæ was commonly used in Germany, but it was difficult to obtain in this country. In fact when I was a student in 1865 I wished to make some observations on sugar in urine, and was unable to get liquor sodæ, and had to make my Fehling's solution with liquor potassæ instead. On account of this difficulty Pavy substituted liquor potassæ for liquor sodæ, and for many years the solution thus modified went by his name. But he introduced another test in which not only the cupric hydrate in the solution, but also the cuprous hydrate produced by reduction, are kept in solution by means of ammonia so that no precipitate occurs on boiling, and the occurrence of reduction is rendered evident only by a loss of the blue colour.

TESTS FOR BILE ACIDS.

(1.) *Sulphur Test.*—Bile and the salts of biliary acids counteract the repulsion between water and fats, so that if a greasy surface be wetted with bile or a solution of bile salts, water subsequently applied to it no longer stands in drops on the surface, but sets it evenly. This power is much used in water-colour painting, and is probably of very considerable physiological importance in regard to the absorption of fats. This power of bile to lessen surface tension exists in relation to other substances as well as fats. Gently sprinkle a little finely powdered sulphur on the surface of urine; it will lie there for a long time unwetted, and does not sink. If bile be present in the urine it soon sinks. Sprinkle some lycopodium on the surface of the urines, and a similar result will follow though slowly.

(2.) *Pettenkofer's Test.*—To about 2 inches of urine in a test-tube add three or four drops of syrup or a piece of cane-sugar the size of a small pea. Shake the tube so as to mix thoroughly and dissolve the sugar if it be used in the solid state. Pour out on to a white plate as much as will cover a space of an inch or an inch and a half in diameter, and then let three or four drops of strong sulphuric acid fall close to it. Incline the plate so as to let them mix. If bile be present, a purple ring will form at the place of contact.

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